The Journal of Animal & Plant Sciences, 31(3): 2021, Page: 894-899
ISSN (print): 1018-7081; ISSN (online): 2309-8694

Short Communication

POTENTIAL OF FUNGAL ENDOPHYTES TO ANTAGONISE *PUCCINIA STRIIFORMIS* CAUSING WHEAT YELLOW RUST

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ABSTRACT

Intense exploitation of pesticides has instigated agricultural and environmental hazards. Microbial inoculation is an alternate to pesticides for antagonising pathogens and is an environmental friendly approach. In this study, we evaluated the potential of fungal endophytes of desert plants, to control the yellow rust of wheat (*Triticum aestivum* L.) caused by *Puccinia striiformis*. Four endophytes i.e. *Colletotrichum lindemuthianum, Piriformospora indica*, *Acremonium lolii* and *Trichoderma viride* were selected *in vitro*, on the basis of their positive impact on the growth of wheat seedlings. Seeds of two rust susceptible wheat genotypes namely Fareed-06 and Shafaq-06 selected by screening experiment against the disease were treated with spore suspensions of these four endophytes separately using randomized complete block design under factorial arrangement. The data concerning area under disease progress curve (AUDPC), final disease severity (FDS), coefficient of infection (CI), thousand grains weight (TGW) and grain yield (GY) was recorded. Results showed that endophytic inoculated susceptible wheat genotypes exhibited the tolerance against the *Puccinia striiformis*. The endophyte *P. indica* significantly decreased the final disease severity and area under disease progress curve, resulting an increase in the grain yield 12.2% in the Fareed-06 and Shafaq-06 followed by the endophytes *T. viride, C. lindemuthianum* and *A. lolii* as 10.6%, 06.2% and 04.2% respectively. The present study concluded that fungal endophytes are valuable microbes which can be employed to induce tolerance against *P. striiformis* and yellow rust for better and sustainable wheat production.

Keywords: *Piriformospora indica*, endophytes, disease severity, grain yield, tolerance

https://doi.org/10.36899/JAPS.2021.3.0278

INTRODUCTION

Wheat (*Triticum aestivum L.*) is widely cultivated cereal crop in the majority of the world regions and is the vital and essential staple food of 36% world's population (2 billion people) (USDA, 2017). The food security is highly focused on obtaining more food to fulfil the needs of burgeoning population which can only be accomplished when the production of wheat as well as other cereal crops is increased globally. The wheat yield is badly affected by many of the abiotic and biotic maladies (Jellis, 2009). Among biotic factors, diseases badly affect wheat yield in which rusts have caused huge yield losses and significant damage in recent years. Wheat rusts are prevalent throughout the world and its new races are evolving unremittingly day by day and infecting resistant varieties (Waqar *et al.,* 2018).

Yellow rust of wheat caused by basidiomycete fungus *Puccinia striiformis* is the most significant wheat disease worldwide. Owing to its economic and social losses, it is presently unsympathetic threat to the global food security (Hovmøller *et al.,* 2011). Susceptible genotypes show the appearance of yellow streaks on the leaves, followed by elongated, bright yellow, small pustules arranged in obvious rows on leaf sheaths, awns and glumes resultantly spores accumulation within the florets and on developing grain. Seed produced from rust damaged crop express low vigour and poor emergence after germination. Wheat yield losses of 10-70% depend on initial infections, susceptibility of the genotypes, inoculum density and its multiplication rate (Chen, 2005). Early infection causes 100% yield loss on susceptible genotypes whereas disease persists to develop during the whole growing season (Chen, 2013).

To minimize the severe wheat yield losses by different pathogenic diseases and abiotic stresses there is need to find out alternative and viable approaches for growing better and sustainable food with less detrimental environmental impacts. Among these approaches, biological control of pathogens by using endophytes is a cost effective and environment safe approach. Endophytes are metabolically active fungal or bacterial microbes with internal colonization, inconspicuous infections, transiently symptomless and diverse life history strategies (Hardoim *et al.,* 2015; Irabor and Mmbaga, 2017). The beneficial impact of endophytes to plant diseases have amplified the concern of farmers and researchers for augmenting agricultural production.

Endophytes antagonise disease pathogens directly by hyperparasitism, production of lytic enzymes and antimicrobial metabolites, indirectly by inducing systemic resistance, contending for space and nutrients and endorsing plant growth (Kavamura *et al.,* 2013; Mmbaga *et al.,* 2018). In our previous research, numerous fungi that colonized different desert plants endophytically have been screened to evaluate their potential to promote wheat growth and to combat leaf rust pathogens. Four of the selected fungal endophytes displayed great potential in controlling *Puccinia recondita* causing wheat leaf rust in field conditions (Anwaar *et al.,* 2019). Based on the background we assume that fungal endophytes can be treated against the yellow rust caused by *Puccinia striiformis.* So, in present study, we screened several fungal endophytes and investigated the antagonistic capacities of the four selected isolates against *Puccinia striiformis* to wheat susceptible genotypes in artificially inoculated diseased conditions.

MATERIALS AND METHODS

Screening of wheat germplasm against yellow rust: Fifty local genotypes of wheat were sown for screening to yellow rust in the research field area of University of Agriculture, Faisalabad during December, 2015. Each genotype was planted in experimental plot size of 1.2 m x 2.5 m surrounded by three rows of highly susceptible genotype Morocco. Rust inoculation methods like needle injection, rubbing, spraying and dusting with talcum powder were exploited at tillering and heading stage on Morocco for the development of a heavy pressure of rust infection (Hussain *et al.,* 2015; Anwaar *et al.,* 2019)

Isolation of Fungal Endophytes: Samples of naturally occurring healthy roots, leaves and stems were taken at random from the various desert locations of Thar, Cholistan and Rohi and were shifted to the mycology and genetics lab through ice bucket for isolation of endophytes. Samples were sterilized, incubated for the emergence of endophytic fungi, purified and identified by the techniques described previously (Anwaar *et al.,* 2019).

Selection of efficient wheat compatible endophytic fungi: Spores of many endophytic fungi were harvested in distilled water by rubbing the surface of a sporulating pure culture with a sterile bent glass rod and maintained the spore suspension of 1×10^6 ml/L by dilution method. Germinating wheat seed were kept in test tubes containing 0.3% agar concentration in distilled water with fungal spore suspension and incubated at 25° C. After the suitable intervals, root and shoot length of wheat seedlings were measured for investigating the fungal endophytes. Consequently, four best endophytes were selected for further experimentation (Anwaar *et al.,* 2019)

***In-Vivo* Potential of Fungal Endophytes:** Seeds of two yellow rust susceptible wheat genotypes Fareed-06 and Shafaq-06 were soaked separately for 24 h in spore suspensions of four nominated (from lab experiments) efficient and compatible endophytes. The fungal endophytes *Colletotrichum lindemuthianum, Piriformospora indica*, *Acremonium lolii* and *Trichoderma viride* were applied to the seeds of the genotypes of Fareed-06 and Shafaq-06. During last week of November, 2015, these endophytic treated seeds were sown under randomized complete block design through factorial arrangement repeated thrice and untreated as control. Rust inoculation was done artificially at tillering and heading stage as performed in prior screening experiment. The FDS (%), AUDPC value, 1000 grain weight (g), Grain yield (gm^{-2}) and yield increased (%) were measured for assessing the potential of fungal endophytes against yellow rust fungus *P. striiformis* as well as their symbiotic response for rust susceptible genotypes in disease vulnerable conditions. The endophytes were re-isolated and identified from the inoculated host plants to confirm the colonization of the fungal endophytes within tissues.

Data Recording of Yellow Rust: Rust severity in percentage and host response was recorded by modified Cobb's scale described by Peterson *et al.* (1948). Rust severity was recorded four times with the consecutive gap period of 10 days while Morocco expressed 40-50% severity. Rating of the final disease severity (FDS) was noted as Morocco showed 90-100% severity. Coefficient of infection (CI) values were calculated for the rating of host responses of susceptible (S), resistant (R), moderately susceptible (MS) and moderately resistant (MR) (Pathan and Park, 2006). For each genotype, area under disease progress curve (AUDPC) with 10 days interval was calculated by the following equation (Pandey *et al.,* 1989).

$$\mathbf{AUDPC = d\ [1/2\ (y_1 + y_k) + (y_2 + y_3 + - - - - - + y_{k-1})]}$$

Where, d= days between two consecutive records (time intervals)

$y_1 + y_k$ = Sum of the 1st and last disease scores

$y_2 + y_3 + - - - - - + y_{k-1}$= Sum of all in between disease scores.

Statistical analysis: Collected data of different parameters of FDS (%), AUDPC value, 1000 grain weight (g), Grain yield (gm^{-2}) and yield increased (%) were analysed using two way analysis of variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT), Tukey's test at 5% probability level in screening experiment and Least Significant Difference (LSD) test for other experiment (Steel *et al.,* 1997).

RESULTS

Susceptibility of Wheat Genotypes against Yellow Rust: The yellow rust significantly affected the local wheat genotypes presenting final disease severity ranged from 20-80% (Table 1). The genotypes of Shafaq-06, Fareed-06, MH-97 and Chenab-00 exhibited the highest of 80% final disease severity followed by Inqalab-91 and Aas-02 (Table I). Correspondingly, the highest values of AUDPC and CI were recorded in genotypes of Fareed-06 (1463, 76.0), MH-97 (1413, 76.0), Shafaq-06 (1400, 76.0), Inqalab-91 (1367, 76.0), Chenab-00 (1283, 76.0) and Aas-02 (1283, 78.0), respectively and were entitled as susceptible, whereas Faisalabad-08 and Bhakhar-08 both depicted the same CI values of 8.0, and also the lowest AUDPC values of 200 and 265, thus, were highly resistant to the yellow rust pathogen (*Puccinia striiformis*). The rest of the genotypes were ranked as moderately resistant to susceptible depicting the range of values 300 to 465 for portraying their resistance as well as 475 to 850 susceptibility (Table 1).

Table 1. Impact of wheat yellow rust on final disease severity, area under disease progress curve and coefficient of infection in field conditions.

Genotypes	FDS (%)	AUDPC	CI	IR
Aas-02	80	1283	78	S
Chenab-00	80	1283	76	S
Fareed-06	80	1463	76	S
Inqalab-91	80	1367	76	S
MH-97	80	1413	76	S
Shafaq-06	80	1400	76	S
Kohistan-97	70	875	64	S
Punjab-11	70	1042	63	S
SH-02	70	875	64	S
Shaheen-94	70	983	64	S
Watan-92	70	945	58	S
Faisalabad-83	60	825	51	S
Hashim-10	60	925	51	S
Moomal-02	60	825	50	S
Parsab-08	60	825	50	S

FDS= Final disease severity, AUDPS= Area under disease progress curve, CI= Coefficient of infection, IR= Infection response

Impact of Fungal Endophytes on Disease Tolerance: From diverse endophytes, the four endophytes namely *Acremonium lolii, Colletotrichum lindemuthianum*, *Piriformospora indica,* and *Trichoderma viride* were designated as efficient and compatible for potentially aggressive against *Puccinia striiformis*, derivative of *in-vitro* evaluation. Endophytic impact was appraised on yellow rust susceptible genotypes Shafaq-06 and Fareed-06 in disease conditions pacing artificially. These susceptible genotypes revealed significant outcomes posing with fungal endophytes.

Endophytic inoculation as seed priming improved thousand grains weight and grain yield by falling the FDS and AUDPC values of yellow rust susceptible genotypes in rust vulnerable conditions comparative to control. Fungal endophytes abridged disease severity effectually as 40% FDS was witnessed in *Piriformospora indica* followed by *Trichoderma viride* (50%), *Colletotrichum lindemuthianum* (55%) and *Acremonium lolii* (60%), respectively (Table 2). Amid the examined genotypes, Fareed-06 indicated slightest FDS (56%) than that of Shafaq-06 (58%). Likewise, least AUDPC value (625.0) was observed in *P. indica* followed by *Trichoderma viride* (800.0), *Colletotrichum lindemuthianum* (933.3) and *Acremonium lolii* (1104.2), comparative to control condition (no endophytic inoculation) to the susceptible wheat genotypes (Table 2).

Symbiotic impact of fungal endophytes to susceptible wheat genotypes and potentially aggressive input for *Puccinia striiformis* were contributed significantly for alleviating wheat plant to yellow rust through antagonising the disease attack by subsiding disease severity consequently enhanced thousand grain weight and grain yield (Table 2). *P. indica* indicated significant performance by increasing 12.3% grain yield comparing to control followed by *T. viride* 10.6 %, *C. lindemuthianum* 6.2% and *A. lolii* 4.2% in yellow rust conditions. Both grains weight and yield were effectually improved by the symbiosis of *T. viride* and *P. indica* while the *C. lindemuthianum* and *A. lolii* revealed moderate performances for conferring tolerance against *Puccinia striiformis*.

Numerous mechanisms of fungal endophytes have been described in suppressing the growth of pathogens. Endophytic microorganisms induce defence mechanisms and systemic acquired resistance in host plants by synthesizing different antibiotic substances, secondary metabolites, bioactive and volatile Organic Compounds (VOCs) to counteract pathogen attack by hampering growth of pathogen (Wang *et al.,* 2009; Tomsheck *et al.,* 2010; Joseph and Priya, 2011). A competition for host resources and space may also take place between attacking pathogens and already existent endophytes (Arnold and Lutzoni, 2007; Wang *et al.,* 2007). *P. indica* exhibited paramount valuable outcomes followed by *T. viride, C. lindemuthianum* and *A. lolii* for prompting tolerance against *P. striiformis*. These fungal endophytes from desert plants were utilized for the improvement of wheat plant growth and yield under yellow rust disease stress. Exploitation of these fungal endophytes ascertained extremely efficacious in enriching the yield of susceptible genotypes (Fareed-06 and Shafaq-06). Findings of current study validated better grains weight and yield interconnected principally to the decrease in disease severity due to the endophytic association with wheat host. Enlarged photosynthetic area and net assimilation efficiency of host inferred the potentially antagonistic and role of fungal endophytes in plant health. The decrease in disease severity details the larger surface area for producing and partitioning of photo assimilates towards reproductive growth, thus, better grains weight and yield. The potential of fungal endophytes against yellow rust fungus *P. striiformis* contributed substantially for stabilizing susceptible wheat host under disease attack by stimulating disease tolerance. Alike, Rodriguez *et al.* (2009) and Suryanarayanan *et al.* (2009) informed antagonistic role of *C. lindemuthianum* in *Solanum lycopersicum* with augmented disease resistance and improved growth and biomass as witness in the current study. Numerous studies conveyed the antagonistic role of *T. viride* as well as other *Trichoderma* spp. for prompting positive impact on the host plants and fighting with diverse diseases (Mastouri *et al.,* 2010; Montero-Barrientos *et al.,* 2010; Shoresh *et al.,* 2010).

Table 2. Potential of fungal endophytes confronting fungus of wheat yellow rust *P. striiformis* in field conditions.

Factors	**FDS**	**AUDPC**	**TGW**	**GY**	**YI**
Genotypes (G)					
Fareed-06	56.0A	961.6A	36.2B	256.8B	7.17B
Shafaq-06	58.0A	953.3A	38.1A	276.2A	9.41A
Endophytes (E)					
Control	80.0A	1325.0A	34.5E	248.1C	0.00E
C.lindemuthianum	55.0BC	933.3BC	36.6C	264.5B	6.15C
A. lolii	60.0B	1104.2B	35.9D	259.0B	4.18D
T. viride	50.0BC	800.0C	38.8B	278.0A	10.61B
P. indica	40.0C	625.0D	40.0A	283.0A	12.25A
LSD ($p \leq 0.05$)					
G	11.9	109.9	0.3367	3.5703	0.7570
E	18.9	173.9	0.5324	5.6451	1.1970
G × E	26.7	245.9	0.7529	7.9834	1.6928
F-value					
G	30.00^{NS}	521^{NS}	28.6163**	2842.13**	23.941**
E	1320.00^{**}	438771**	29.5270**	1201.28**	146.476**
G × E	30.00^{NS}	10625^{NS}	0.5963*	56.72^{NS}	4.550**

FDS= Final disease severity, AUDPS= Area under disease progress curve, TGW= Thousand grains weight, GY= Grain yield, YI= Yield increase

In a previous study Anwaar *et al.* (2019) found these four fungal endophytes *P. indica, T. viride, C. lindemuthianum* and *A. lolii* paramount valuable for prompting tolerance against fungus *P. recondite.* They reported that endophytes decreased the leaf rust (up to 30-60%) and decreased area under disease progress curve (AUDPC), resultantly achieved the 12.2% 10.6%, 06.2% and 04.2% grain yield gain, respectively. According to Rabiey and Shaw (2016) *P. indica* exploitation in wheat decreased 70% disease severity of Fusarium head blight, improved the grains weight and yield. The average rise of 24.2 and 17.3% for thousand grains weight and grain yield was observed. In another study by Rabiey (2015), *P. indica* employment at sowing time decreased the disease severities of septoria leaf blotch, powdery mildew and yellow rust by 65, 63, and 29%, respectively. Subsequently, it also improved wheat grain yield by 27, 48 and 25%, respectively. Thus, deeming the substitute of chemical fungicide, fungal endophytes utilization is expedient in getting better and sustainable wheat yield from yellow rust vulnerable areas.

Conclusion: The study conclude that fungal endophytes can protect *Triticum aestivum* (bread wheat) from plant pathogenic fungus *P. striiformis* by declining the disease severity and improving the grain yield in field. The symbiotic association of *P. indica* followed by *T. viride, A. lolii* and *C. lindemuthianum* with wheat host plant can induce tolerance against the disease.

Acknowledgements: Authors appreciatively acknowledge the Bahauddin Zakariya University (BZU), Multan and University of Agriculture, Faisalabad (UAF) for providing facilities to conduct this research.

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